Supplemental Materials

Epitope mapping of monoclonal antibodies against tTG. Several commercial monoclonal antibodies including cub7401 (1), cub7402 and TG100 (*Labvision*, CA) and 4C1 (2)(Hybridoma Bank, U. of Iowa) were examined for their epitope on TTG. This is an essential step in studying structure and function of TTG as these antibodies have been used extensively to probe tTG's function (3, 4). As site-directed mutagenesis is a powerful tool in epitope mapping, we used a series of C-terminal truncation and point mutation mutants available in the laboratory in localizing the epitope (**supFig. 2**). C-terminal truncation mutants including ΔS538, ΔE447, ΔP345 and ΔV228 were purified as GST fusion proteins as described (**supFig. 2**)(5). The ΔS538 mutant is similar in size to a TTG homologue (designated as tTGH) expressed in human erythroleukemia cells (6). The ΔE447 mutant lacks the putative calcium binding domain (amino acids 446-453). The ΔP345 was designed to remove additional charged residues that could play a role in GTP and ATP binding. The ΔV228 mutant stopped at intron-exon boundaries for exon 4. In addition to TGase active site mutant, C277A, and GTP/ATPase site mutant K174L, we also constructed a series of cysteine substitution mutants including C290S, C336S, C371S located in catalytic core region and C510S and C524S located in the C-terminal β-barrel region.

Initially, tTG and C-terminal truncation mutants were used to localize the epitope of these antibodies. All antibodies reacted with full-length tTG and Δ538 but not with ΔE447, ΔP345 and ΔV228 indicating that the epitope is localized between amino acid # 447 and # 538 (**supFig. 3**). Additional immunoblots analysis using point mutation mutants indicated that amino acid # 451 to 454 (+off4) are important for the epitope of cub7401, cub7402 and 4C1 (**supFig. 3**), and the amino acid # 456-464 (PR_1) are important for the epitope of TG100 (sup**Fig. 3**). It is interesting that the amino acid residues critical for the recognition of these antibodies are all localized in the exposed loop region (**residue # 455-478**). Based on these results, Cub7401 and Cub7402 were used in the Immunoblot in this study. *The localization of the epitope ensures the correct interpretation of gene expression results obtained from using these antibodies.*

Validation of Taqman PCR probes and primers. To validate taqman real-time PCR primers and probes used in this study, cDNAs prepared from HUVEC were used as template for real-time PCR amplification of mRNAs encoding tTG, tTG_{V1} and tTG_{V2}. After PCR reactions, reaction products were analyzed using high resolution agarose (ultrapure 1000, Invitrogen) gel electrophoresis. As predicted, 90bp, 99 bp and 75 bp bands were amplified from PCR reactions amplifying mRNAs encoding tTG, tTG_{V1} and tTG_{V2}, respectively (**supFig. 4**)(see Main text for primers and probes sequences).

Sup-References

1. Birckbichler, P. J., Upchurch, H. F., Patterson, M. K., Jr., and Conway, E. (1985) A monoclonal antibody to cellular transglutaminase. *Hybridoma* 4, 179-186
2. Lesort, M., Tucholski, J., Zhang, J., and Johnson, G. V. (2000) Impaired mitochondrial function results in increased tissue transglutaminase activity in situ. *J Neurochem* 75, 1951-1961
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Supplemental Figure Legends

Sup-Figure 1. Alignment of nucleotide sequences. The sense strand of cDNA sequences from nucleotides # 1901-2901 (NM_004613) is shown. The sequences that are deleted in tTG_{V1} and tTG_{V2} are displayed as "------". The boxed sequences represent the location of the spliced location. TaqMan assay primers are **underlined** and probes targeting tTG, tTG_{V1} and tTG_{V2} mRNA are colored in blue, red, and green, respectively. Note that the TaqMan probes targeting tTG_{V1} and tTG_{V2} mRNA overlap part of the sequences flanking the splicing location. " / " denotes the junction between exons XII and XIII in tTG.

Sup-Figure 2. Structure and functional domains of tTG and mutants used for epitope mapping. The functional domains of tTG including N-terminal β-sandwich (residue 1 to 139), The catalytic core region (140-454), and two C-terminal beta-barrel 1 (479-585) and barrel 2 (residue 586-687. The TGase active site triad residues (Cys277, His335, and Asp358). Potentail GTP binding sites including 233DXXG235 (GTP-1) and 386TKXD389 (GTP-4) and an exposed loop mutant, 456FXXXK464 are illustrated (PR_1) There are surface charge mutants located at residues 363EKSE366 (+off2), 451EERE454 (+off4) and 467EKEE470 (+off5). On the bottom figure, several C-terminal truncation mutants are also illustrated.

sup-Figure 3. Epitope mapping of mouse monoclonal antibodies using C-terminal truncation and point-mutation mutants. A. Left top panel, Coomassie blue staining of affinity purified GST-fusion proteins. 3 μg/lane. Bottom panel, Immunoblot was developed using mouse monoclonal 7401. The same results were obtained using cub 7402, TG100 and 4C1. 100 ng/lane. **B.** Different point mutation mutants (100 ng/lane) were separated on 9% SDS-PAGE and developed using cub 7401 (top panel) or TG100 (bottom panel). The same results were obtained using Cub 7401, Cub7402 and 4C1.

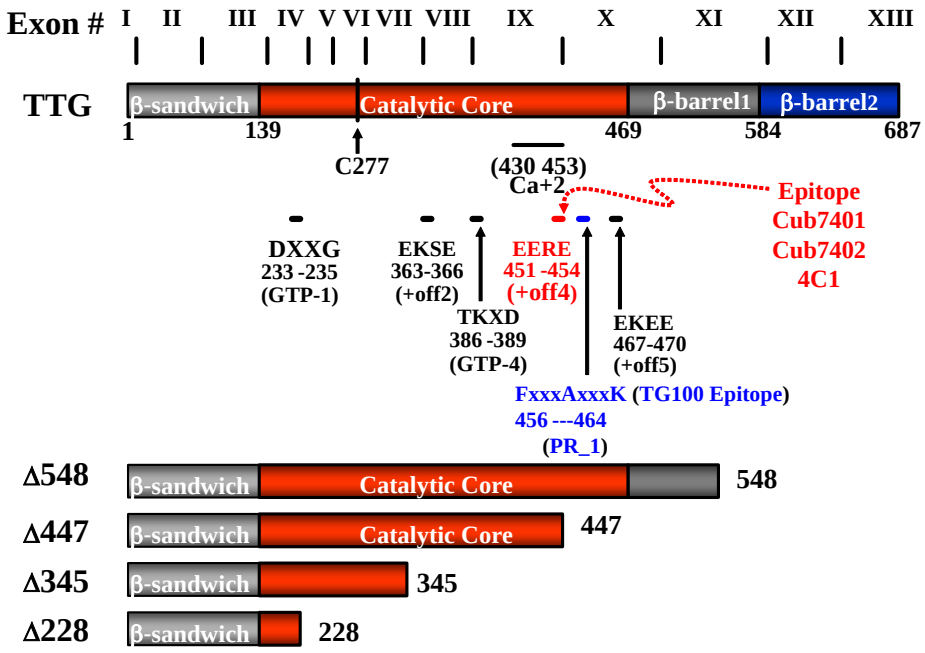
sup-Figure 4. Gel Electrophoresis of the reaction products after real-time PCR. Taqman real-time PCR assays were performed as described in the text. Oligo-dT primed cDNA were prepared from total RNAs from HUVEC and used as templates in PCR reactions containing Taqman's PCR primers and probes for tTG, tTG_{V1} and tTG_{V2}. The PCR conditions were described in Materials and Methods section. After PCR reaction, the amplification products were electrophoresed on a 2% agarose (ultrapure agarose 1000, Invitrogen, CA) gel with 50 bp DNA ladder as DNA molecular weight marker.

Sup-Figure 5. Effects of tTG, tTGV1, and tTGV2 on cell viability and apoptosis of HEK 293 cells. Transfections were performed quadruple in 24-well plates as described under *Methods*. LDH release, cell viability and caspase 3/7 activation were determined 22, 46, and 72 hours after transfection as describered under *Methods*. Growth media was used as background value in these assays. **A.** LDH release assay; **B**. Cell Viability assay; **C**. Caspase 3/7 activatiuon assay.


1901 CGCAAGCTGGTGGCTGAGGTGTCCCTGCAGAACCCGCTCCCTGTGGCCCTGGAAGGCTGCACCT/TCACTGTGGAGGGGCCGGCCTGACTGAGGAGCAGA tTG
1901 CGCAAGCTGGTGGCTGAGGTGTCCCTGCAGAACCCGCTCCCTGTGGCCCTGGAAGGCTGCACCT- ---------- tTGv1
1901 CGCAAGCTGGTGGCTGAGGTGTCCCTGCAGAACCCGCTCCCTGTGGCCCTGGAAGGCTGCACCT- ---------- tTGv2
2001 AGACGGTGGAGATCCCAGACCCCGTGGAGGCAGGGGAGGAAGTTAAGGTGAGAATGGACCTGCTGCCGCTCCACATGGGCCTCCACAAGCTGGTGGTGAA tTG
1965 ---------- tTGv1
1965 ---------- tTGv2
2101 CTTCGAGAGCGACAAGCTGAAGGCTGTGAAGGGCTTCCGGAATGTCATCATTGGCCCCGCCTAAGGGACCCCTGCTCCCAGCCTGCTGAGAGCCCCCACC tTG
1965 ---------- tTGv1
1965 ---------- tTGv2
2201 TTGATCCCAATCCTTATCCCAAGCTAGTGAGCAAAATATGCCCCTTCTTGGGCCCCAGACCCCAGGGCAGGGTGGGCAGCCTATGGGGGCTCTCGGAAAT tTG
1965 ---------- tTGv1
1965 ---------- tTGv2
2301 GGAATGTGCCCCTGGCCCATCTCAGCCTCCTGAGCCTGTGGGTCCCCACTCACCCCCTTTGCTGTGAGGAATGCTCTGTGCCAGAAACAGTGGGAGCCCT tTG
1965 ---------- tTGv1
1965 ---------- tTGv2
2401 GACCTTGGCTGACTGGGGCTGGGGTGAGAGAGGAAAGACCTACATTCCCTCTCCTGCCCAGATGCCCTTTGGAAAGCCATTGACCACCCACCATATTGTT tTG
1965 ---------- tTGv1
1965 ---------- tTGv2
2501 TGATCTACTTCATAGCTCCTTGGAGCAGGCAAAAAAGGGACAGCATGCCCCTTGGCTGGATCAGGGAATCCAGCTCCCTAGACTGCATCCCGTACCTCTT tTG
1965 ---------- tTGv1
1965 ---------- tTGv2
2601 CCCATGACTGCACCCAGCTCCAGGGGCCCTTGGGACAGCCAGAGCTGGGTGGGGACAGTGATAGGCCCAAGGTCCCCT/CCACATCCCAGCAGCCCAAGCT tTG
1965 ---------- CCACATCCCAGCAGCCCAAGCT tTGv1
1965 ---------- ---------- tTGv2
2701 TAATAGCCCTCCCCCTCAACCTCACCATTGTGAAGCACCTACTATGTGCTGGGTGCCTCCCACACTTGCTGGGGCTCACGGGGCCTCCAACCCATTTAAT tTG
1987 TAATAGCCCTCCCCCTCAACCTCACCATTGTGAAGCACCTACTATGTGCTGGGTGCCTCCCACACTTGCTGGGGCTCACGGGGCCTCCAACCCATTTAAT tTGv1
1965 ---------- tTGv2
2801 CACCATGGGAAACTGTTGTGGGCGCTGCTTCCAGGATAAGGAGACTGAGGCTTAGAGAGAGGAGGCAGCCCCCT/CCACACCAGTGGCCTCGTGGTTATTA tTG
2087 CACCATGGGAAACTGTTGTGGGCGCTGCTTCCAGGATAAGGAGACTGAGGCTTAGAGAGAGGAGGCAGCCCCCT CCACACCAGTGGCCTCGTGGTTATTA tTGv1
1965 ---------- CCACACCAGTGGCCTCGTGGTTATTA tTGv2
2901 GCAAGGCTGGGTAATGTGAAGGCCCAAGAGCAGAGTCTGGGCCTCTGACTCTGAGTCCACTGCTCCATTTATAACCCCAGCCTGACCTGAGACTGTCGGA tTG
2187 GCAAGGCTGGGTAATGTGAAGGCCCAAGAGCAGAGTCTGGGCCTCTGACTCTGAGTCCACTGCTCCATTTATAACCCCAGCCTGACCTGAGACTGTCGGA tTGv1
1991 GCAAGGCTGGGTAATGTGAAGGCCCAAGAGCAGAGTCTGGGCCTCTGACTCTGAGTCCACTGCTCCATTTATAACCCCAGCCTGACCTGAGACTGTCGGA tTGv2

Sup Fig. 1 4

Sup Figure 2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584
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C277
(430 453)
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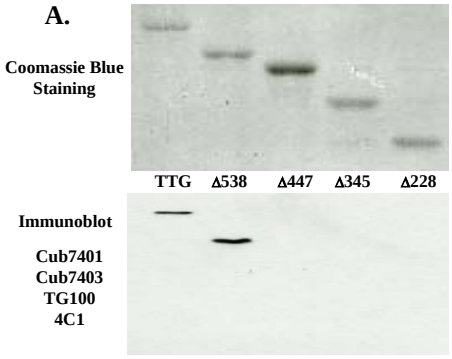
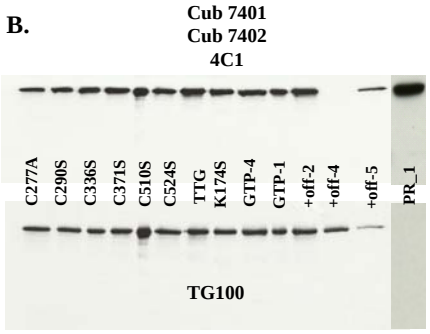
Sup Figure 3





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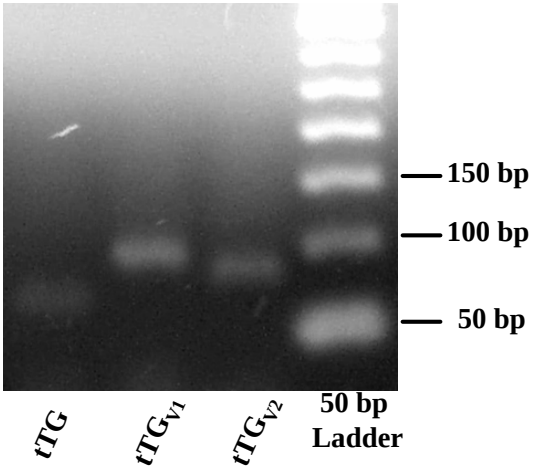

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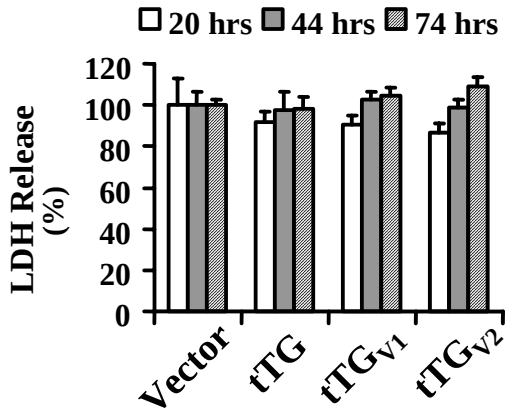

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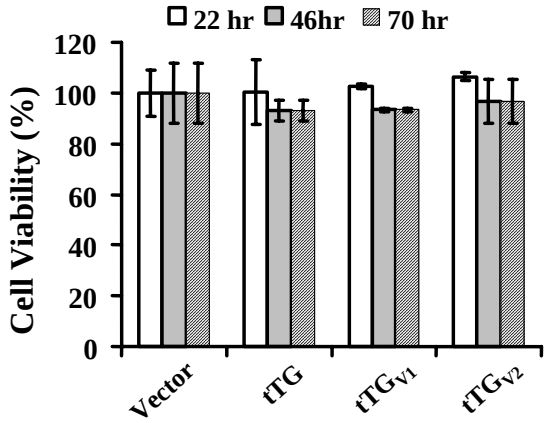
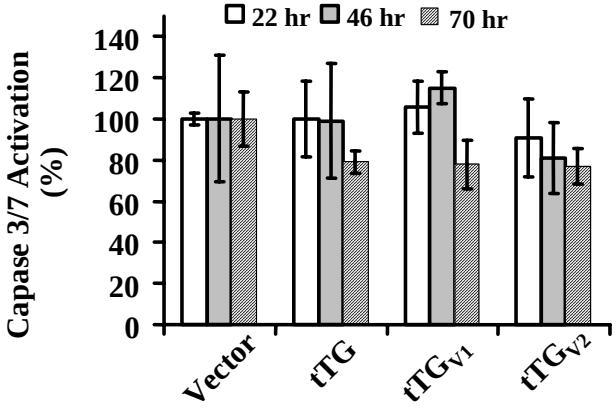
Sup Figure 5B.



Sup-Figure 5B

Sup Figure 5C.



Sup-Figure 5C